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ADAPTATION OF THE FEEDING MECHANISM OF THE OYSTER (*Ostrea gigas*) TO CHANGES IN SALINITY

By A. E. Hopkins

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ADAPTATION OF THE FEEDING MECHANISM OF THE OYSTER *(Ostrea gigas)* TO CHANGES IN SALINITY [1]

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INTRODUCTION

Natural beds of oysters are found characteristically in such inshore waters as the coastal estuaries and bays in which there is a considerable dilution of the ocean water by land drainage. Prytherch (1934), in an extensive monograph describing an experimental study of the ecology of *Ostrea virginica*, concluded that successful propagation of oysters depends upon the presence in the water of copper ions required by the swimming larvae before attachment, or setting, may take place. In nature the copper is introduced into sea water in solution in the fresh water of rivers, thereby locating natural oyster grounds where they are subject to frequent changes in salinity.

Referring to *O. virginica*, Churchill (1920) stated:

> Oysters are found in water ranging in density from 1.002 to 1.025 (about 2.5 to 33.0 parts per mille), but cannot withstand densities lower than 1.007 (9 parts per mille) for indefinite periods. In general they seem to thrive best in densities between 1.011 and 1.022 (14.36 to 28.80 parts per mille).

This range apparently refers to areas in which natural oyster beds are located, or those areas in which propagation takes place.

The feeding activities, however, may not be most efficient at the same range of salinity or density as in the case of propagation. It may frequently be observed that oysters grown on those grounds where seeds are caught most effectively do not fatten as well as those grown in different areas. Oyster growers typically catch seeds in one place and transplant them to localities which experience has shown to be favorable for

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fattening. It appears possible that salinity may be one of the important factors influencing the feeding of oysters.

The gills of oysters, by means of cilia, pump a stream of water from which food particles are filtered and passed along definite grooves to the labial palps, which convey them to the mouth (Nelson, 1923b). The rate at which water is pumped was used by Galtsoff (1928) as a criterion of the rate of feeding under different conditions of temperature. Nelson (1921, 1923a) kept records of the opening and closing of oysters immersed in the open bay along with those of temperature, salinity, turbidity, etc. He concluded that the oyster feeds most rapidly on the flood tide even when the density is approximately the same as during ebb tide. He concluded also that for *O. virginica* a density of 1.008 (10.42 parts per mille) is the lowest at which feeding will go on, and that the minimum density required varies in proportion to that of the water in which the oysters have been grown. His method, however, was to study the opening or closing of oysters under different conditions, judging an open specimen as actively feeding. But, as will be shown below, an oyster may be open without feeding, though clearly when it is closed feeding is impossible.

The mechanism of physiological adaptation to changes in salinity is not the question primarily discussed in this paper. This study deals with the pumping activity of the gills as influenced by salinity, although it is realized that final explanation of the results is a matter of cellular physiology.

MATERIAL AND METHODS

Because of their relatively large size, specimens of *Ostrea gigas* are most favorable material for experimental study. This species is imported from Japan as small seeds and grown in various waters of the Pacific coast. In portions of Puget Sound propagation occurs, and the resulting well-shaped, large oysters were used in these tests.

The method employed has already been described (Hopkins, 1933). A simple lever, resting with the least possible weight upon the upper (right) valve of the specimen, served to record on a kymograph paper the position and movements of the valves, as affected by activity of the adductor muscle. A paper cone, thoroughly waterproofed with a solution of celluloid in acetone, attached to a system of levers made of lightweight straw, was placed so that all water pumped by the gills struck the cone. The recording tip of the straw was made of a sliver of cellophane, and this made its record on the kymograph paper directly below that of the lever recording shell movements. Fixed levers continously recorded the zero positions—the closed position of the shell and the level of cessation of pumping by the gills.

Since the salinity of the water had to be maintained at a constant level, it was necessary to employ a method for continuous aeration and circulation. For this purpose small centrifugal pumps, made of celluloid (Hopkins, 1934) were used. Two of these pumps were used to circulate and aerate the water, without causing any strong currents which might disturb either the oyster or the mechanism for recording the relative rate at which the gills pump. An extra aquarium was interposed between the experimental chamber and the pumps for the control of temperature. In this aquarium either an electric-light bulb or an immersion heater was so controlled that the temperature in the experimental chamber was maintained during all tests at between 17° and 19° C. This temperature level was chosen because it had been previously determined (Hopkins, 1933, 1935) to be most favorable for stability of

the adductor muscle, resulting in the shells remaining at almost their optimum degree of openness. At 20° C. or above the valves tend to become more closed, although the gills themselves pump more rapidly up to between 27° and 28° C.

The salinity of the running water at the laboratory varied generally between 26 and 29 parts per mille. Water of a lower salinity was prepared in a tank, large enough for several changes of water in the experimental aquaria, by mixing the laboratory water with pure spring water. Control tests showed this method to be adequate, for specimens behaved identically in bay water of salinity 28 parts per mille and in water of the same salinity made by mixing spring water and concentrated bay water.

The water level in the experimental tanks was marked so that occasional small additions of spring water prevented more than very slight changes in salinity due to evaporation. The pH of the water varied only slightly, between 7.7 and 8.0, in harmony with that of water on the oyster grounds.

Specimens for experimentation were always kept in the running sea water of the laboratory for several days or weeks before use. During the first one or more days after being placed in the experimental aquarium, a specimen was tested in water of approximately the same salinity as the running laboratory sea water. In making a change of water, of either the same or a different salinity, the water was drained from the experimental tanks, which were then flushed out thoroughly with the new water before being filled to the correct level. To save time, the new water was warmed to the experimental temperature (17°–19° C.) before being siphoned in.

Some specimens were mounted upon a base of plaster of paris and sand, while others were set rigidly upon a small celluloid frame. No ill effect of the plaster of paris was noted.

After completion the kymograph papers were marked off exactly into 5-minute periods for analysis. Planimeter measurements were made of the area enclosed between the record line and the reference line during each 5-minute period. These measurements are considered in the following descriptions as representing S (the degree of openness of the shell) and F (the relative rate of flow of water through the gills) during each 5-minute period.

DESCRIPTION OF EXPERIMENTS

These experiments were started for the purpose of determining whether there is any relationship between salinity and the rate at which water is pumped. It was thought that adaptation to a change in salinity would occur quite quickly, since in their natural environment oysters are subject to frequent and sometimes extreme salinity variations, and it was planned to make a change, allow the pumping mechanism to reach a stable level of activity, then to change again. In this manner it was hoped that sufficient values might be obtained to permit graphical analysis of the relationship between salinity and the rate of feeding.

It soon became evident, however, that such a procedure would require a long period of time, even with only one specimen. Adaptation was found to be extremely slow, and the variations in degree of openness of the valves and in rate of pumping are so great even under conditions of constant salinity and temperature that any test would have to be carried on for many days, or even weeks. It was, therefore, decided to attack the problem by making a study of the process of adaptation following changes in salinity with respect to shell movements and gill activity.

The nature of the results and the different lengths of time during which specimens were subject to water of different salinities make it impossible to present the data obtained in any manner other than by description of the tests with each specimen. Seven such series are described below.

SERIES I

This was the first specimen on which detailed experiments were performed. The salinity at the beginning was 29.40 parts per mille and records were kept for thirty-seven 5-minute periods, or 185 minutes. Then, on the same day, the salinity was lowered to 26.96 parts per mille and the activity recorded for the next 205 minutes, or forty-one 5-minute periods. The records taken at the two salinities (table 1, series I) are closely similar, although the averages in the former case are S, 8.53; F, 6.45; and in the latter case, S, 7.13; F, 5.40. Obviously the results are inadequate for comparison because of the short duration of exposure to each medium.

On the following day the salinity was lowered still further to 24.03 parts per mille and records kept for forty-seven 5-minute periods. The low F values during early treatment at this salinity, as shown in the table, indicate a marked effect of the change, though later values appear to be in harmony with those obtained at the original higher salinities.

The effect of lowering the salinity is more strikingly shown in the tests of the next 2 days (Nov. 9 and 10), after changing the salinity from 24.03 to 17.76 parts per mille. Although during the first day the shell remained approximately as wide open as previously, only a feeble stream of water was pumped by the gills. In figure 1 the consecutive 5-minute values are shown graphically, to illustrate the slow

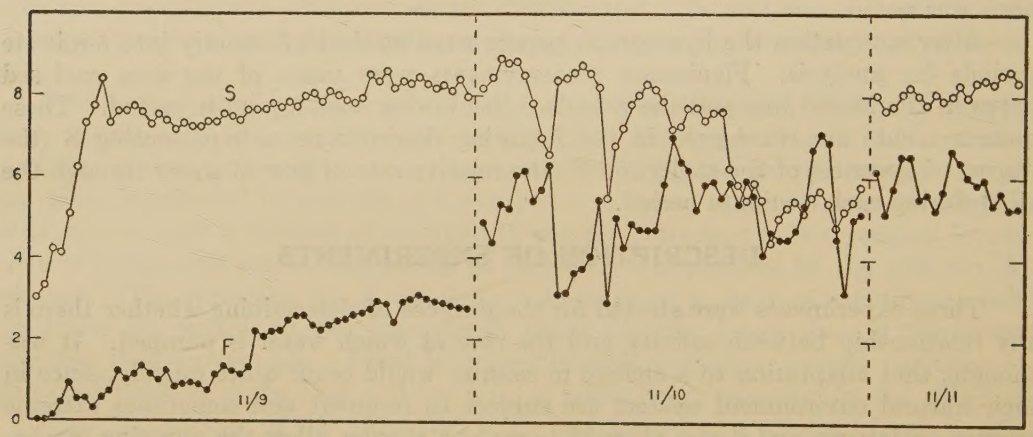

FIGURE 1.—Values of *S* and *F* showing adaptation during 3 days to a salinity of 17.76 p. p. m. following a change from 24.03 p. p. m. Series I. 17°–19° C.

adaptation of the gill mechanism (F) while the valves (S) quickly recovered their normal degree of openness. It is doubtful that adaptation was completed even after more than 2 days. However, the salinity was changed (Nov. 11) to 28.50 to test adaptation to a rise in salinity. Records were kept on the first day for fifty-four 5-minute periods. Within a very short time after the change, the values of F rose almost to normal, though not until the next day did the shell open wide enough to permit completely normal gill activity.

After the specimen had been in this water for 4 days the salinity was again lowered to 22.20 parts per mille. Adaptation to this change was relatively rapid,

occupying but a few hours. On the following day the medium was changed to a salinity of 29.46, and adaptation was almost immediate.

During the preceding tests it appeared that adaptation to a considerable change in salinity is a very slow process, much of which occurs between the recordings on consecutive days. In order to obtain records of as many details as possible of the early stages of adaptation, the salinity was reduced to 14.60 parts per mille and during the following 12 hours records were taken which provided one hundred and twenty-five 5-minute periods. In figure 2 the results are given in detail for the

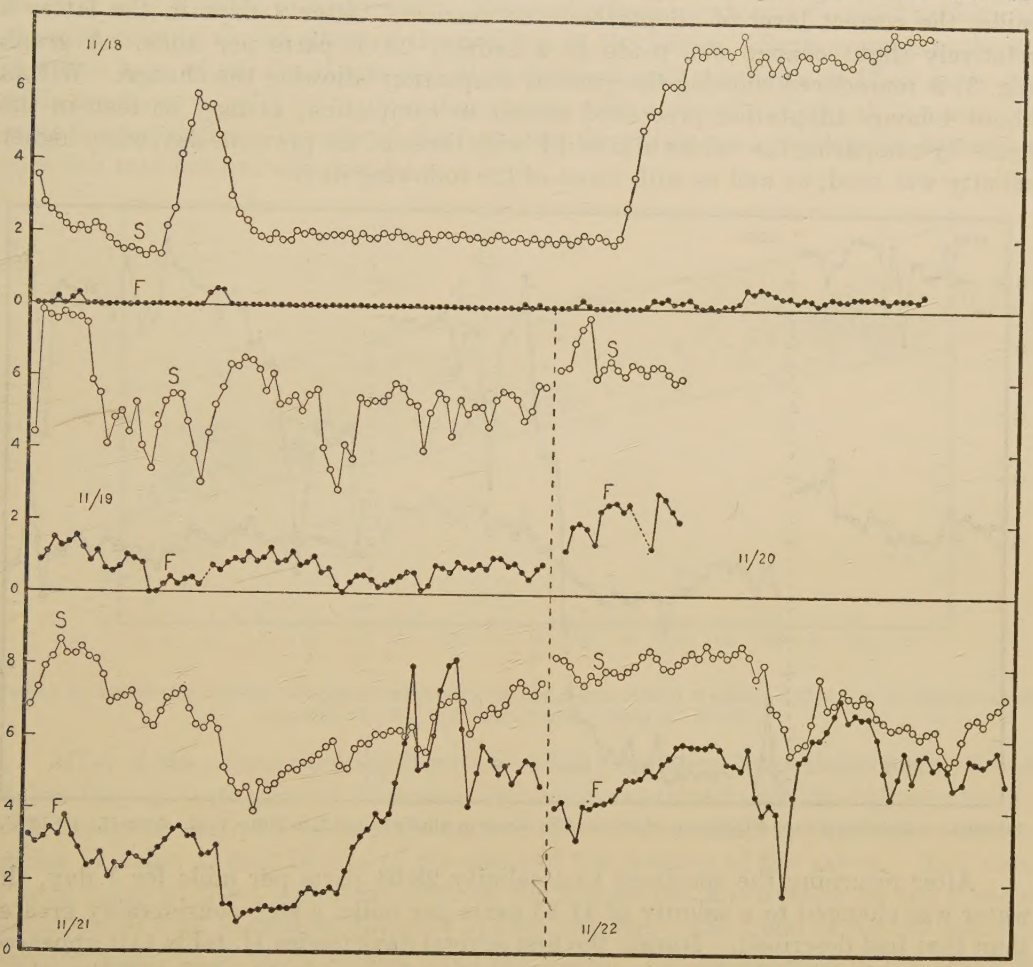

FIGURE 2.—Adaptation to salinity of 14.60 p. p. m. following a change from salinity of 29.46 p. p. m. Series I. Values of *S* and *F* refer to consecutive 5-minute periods. 17°–19° C.

5 days during which this salinity was used. For the first 8 hours (Nov. 18), with minor exceptions, the valves remained relatively close together, and almost no water was pumped. During the following hour the shell consistently became wider open and the gills produced a slight flow of water. Recording was continued for several hours more, but the rate of flow increased very little. However, during the following 4 days the gills steadily recovered, though large variations both in rate of flow and in degree of openness of the valves may be noted. Even after 5 days of treatment recovery could not be called complete, for the values of F never reached the

previous levels. (See series I, table 1.) When the salinity was then raised to 28.56 the specimen became adapted to a comparable level within a few hours.

SERIES II

Preliminary tests in a salinity of 28.87 parts per mille were carried on only during 1 day (thirty-six 5-minute readings), although it later appeared that they should have been continued for several days more (series II, table 1). When the salinity was changed to 22.70 parts per mille for 2 days and then to 28.74 parts per mille, the correct level of adaptation was reached. After 3 days in the latter a relatively slight change was made to a salinity 25.08 parts per mille. A graph (fig. 3) is reproduced showing the gradual adaptation following the change. Within about 4 hours adaptation proceeded almost to completion, as may be seen in the figure by comparing the values of S and F with those of the previous day, when higher salinity was used, as well as with those of the following day.

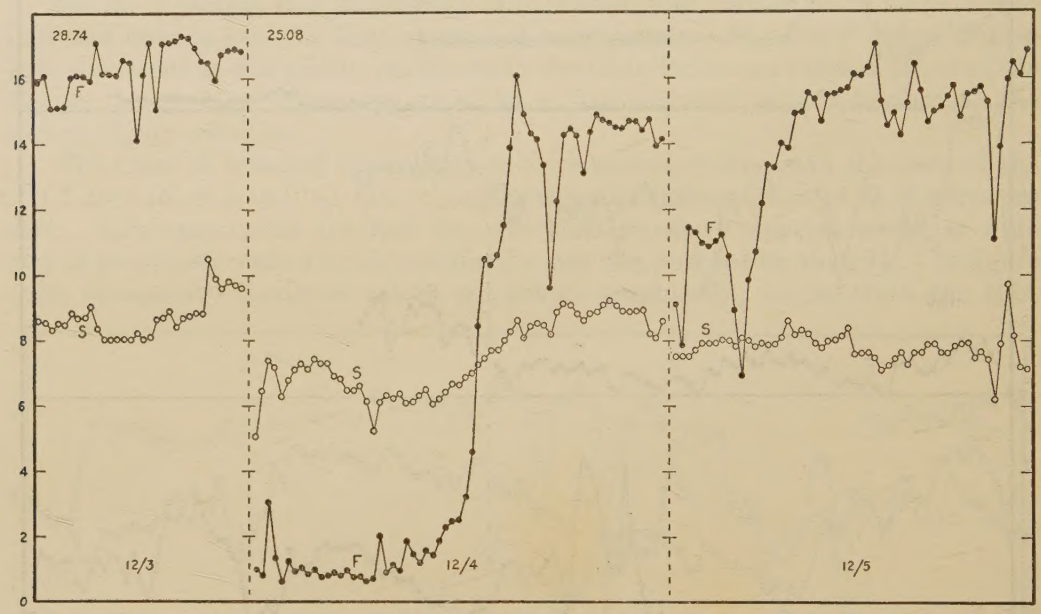

FIGURE 3.—Adaptation of *S* and *F* (5-minute values) following change in salinity from 28.74 to 25.08 p. p. m. Series II. 17°–19° C.

After returning the specimen to a salinity 28.94 parts per mille for 1 day, the water was changed to a salinity of 17.85 parts per mille, a step considerably greater than that just described. During the first several days (series II, table 1) it appeared that adaptation was slowly occurring, but during the remainder of the 11 days there was no further obvious recovery. The valves did not remain open wide enough to permit free flow of water. Yet when the salinity was raised to 27.27 parts per mille recovery was so rapid that activity seemed to be almost at its normal level within 2 or 3 hours.

A considerable difficulty in the analysis of these data lies in the fact that two activities are concerned, namely, the degree of openness of the shell (S) and the rate at which water is pumped (F). The latter is dependent upon the former, and it appears that both functions are influenced independently by changes in salinity. However, even under constant conditions of temperature and salinity, there is a

tremendous variation in the degree of openness. The rate of pumping of water is the activity which primarily is being studied, for on this depends the rate of feeding; but this activity may not be isolated from the influence of the adductor muscle save by propping the valves open; in which case the results would be of little significance with respect to the reactions of the oyster as a whole to environmental factors.

In a previous publication (Hopkins, 1933) it was shown that, at constant temperature, the rate of flow of water through the gills increases as the valves become wider open. By plotting the results of certain series to show the relationship between S and F it is possible to find whether observed effects are due to changed activity of the gills or only to the effect of the position of the valves. Such a graph is shown in figure 4, in which the results for all of the tests with a salinity of 17.85 parts per mille are given in solid points and those taken with a salinity of about 28 parts per mille in open circles. In spite of the fact that the points are considerably dispersed, they fall into relative alinement.

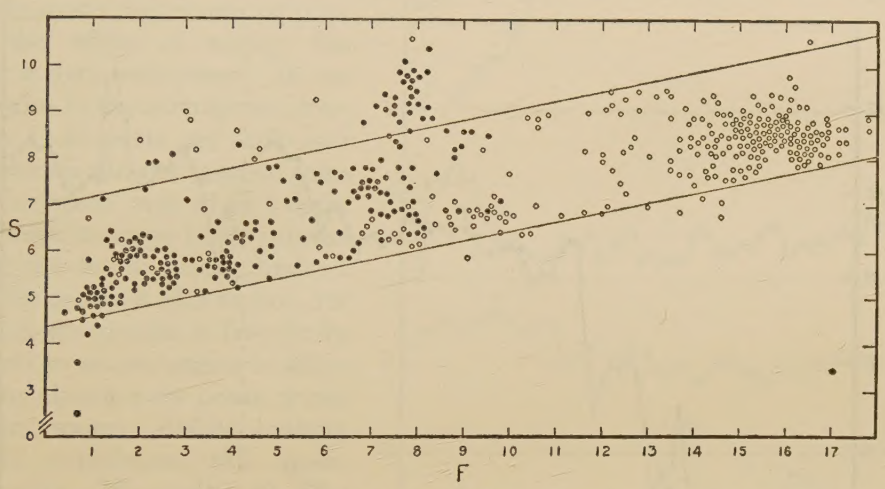

FIGURE 4.—Relationship between *S* (degree of openness of shell) and *F* (rate of flow) at salinity of 17.85 p. p. m. (solid points) as compared with 28 p. p. m. (open circles). Series II. 17°–19° C.

Most of the points resulting from low salinity represent low values of both S and F, but they fall into general alinement with values obtained with the higher salinity. This indicates that at least a large part of the low F readings at the lower salinity (series II, table 1) may be due to the effect of the position of the valves. Yet, also, a good many of these points are high on the S scale but much lower on the F scale as compared with the values obtained at high salinity. The trend of the low salinity curve appears to be much steeper than the other. The significance of the graph is that both the adductor muscle, by controlling shell position, and the gill mechanism were in this case responsible for the lack of complete adaptation to the lower salinity. Tests with other specimens throw further light on the subject.

SERIES III

The preceding two series of tests were concerned with changes in salinity between about 14 and about 29 parts per mille. It was noted that adaptation to a reduction in salinity is quite slow as compared to that following a rise. The question presented itself as to whether this difference was due to the fact that the specimens had been

living in water of the higher salinity (28–29 parts per mille) and consequently any change from this level produced a pronounced effect, while restoration of this level merely permitted normal activity.

The reactions were studied when the specimen was transferred into water of a salinity of 39.10, after 2 days of study at 28.55 and 28.27 parts per mille. Since pure ocean water has a salinity of only 35 to 36 parts per mille, there is no possibility that the specimen could already be adapted to water of even higher salinity. The results of the entire series are summarized in table 1, series III. It may readily be observed that the specimen became adapted quickly to the abnormally high level. In figure 5 the values of S and F are given in detail to show the reactions during the first 2 days in this high salinity. After about 3 hours the specimen was pumping water at a rate only a little below normal, and within the following 4 hours it appeared to become completely adapted, as judged by degree of openness and rate of pumping. Tests of the following day are given on the graph for comparison.

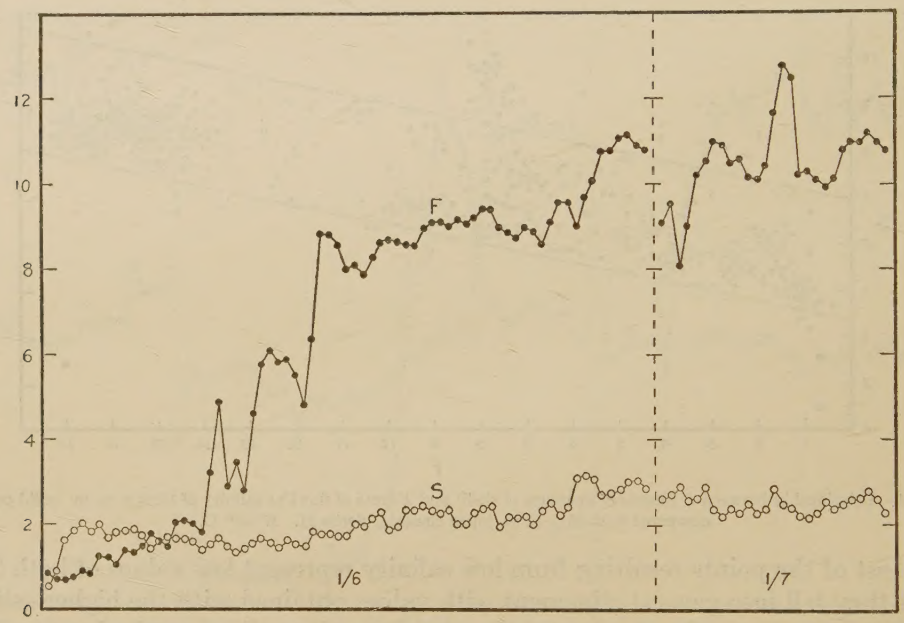

FIGURE 5.—Adaptation of 5-minute values of *S* and *F* to salinity of 39.10 p. p. m. following change from 28.27 p. p. m. Series III. 17°–19° C.

On the other hand, adaptation proceeded at the same slow rate as previously observed when the salinity was lowered to 28.04 parts per mille, as shown in figure 6. At least 3 days were required for restoration of the rate of pumping previously observed, although the valves were much wider open than at the higher salinity. The effect of lowering the salinity in this case appears to be entirely upon the gill mechanism, rather than upon the organs controlling position of the valves.

Since the specimen appeared to function so well in a salinity of 39.10 parts per mille, a short test was made to give a general idea of the upper limit to which it could become adapted. A salinity of 56.32 parts per mille, twice that of the ordinary bay water, was used and records kept for 4 days (series III, table 1). During the first day the shell remained entirely closed, and on the second it opened only to a small degree. However, as the valves became sufficiently far apart the gills were

able to produce a slight flow of water. On the fourth day the valves were abnormally wide open, gaping, although the gills were able to function and produce a slight current. Tests would have been continued save that gaping is generally indicative of loss of tonus of the adductor muscle and death of the organism. However, the oyster was placed in running laboratory water, where, after some days, it apparently recovered completely.

SERIES IV

The experiments described above suggest a marked sensitivity of the oyster to lowered salinity, while the effect of raising the salinity is very temporary. In one case (series I) the salinity was lowered to 14.60 parts per mille and the specimen did not become completely adapted even after 5 days. It is of importance to know approximately the lower salinity limit at which the oyster is able to feed, for in nature the species is frequently subjected to water ranging in salinity from almost pure ocean water to fresh water. Before making such an experiment this specimen (series IV, table 1) was tested for 2 days in water of a salinity of 28.06 parts per mille, after which the salinity was raised to 36 parts per mille and left for 2 days while records were kept to show the progress of adaptation.

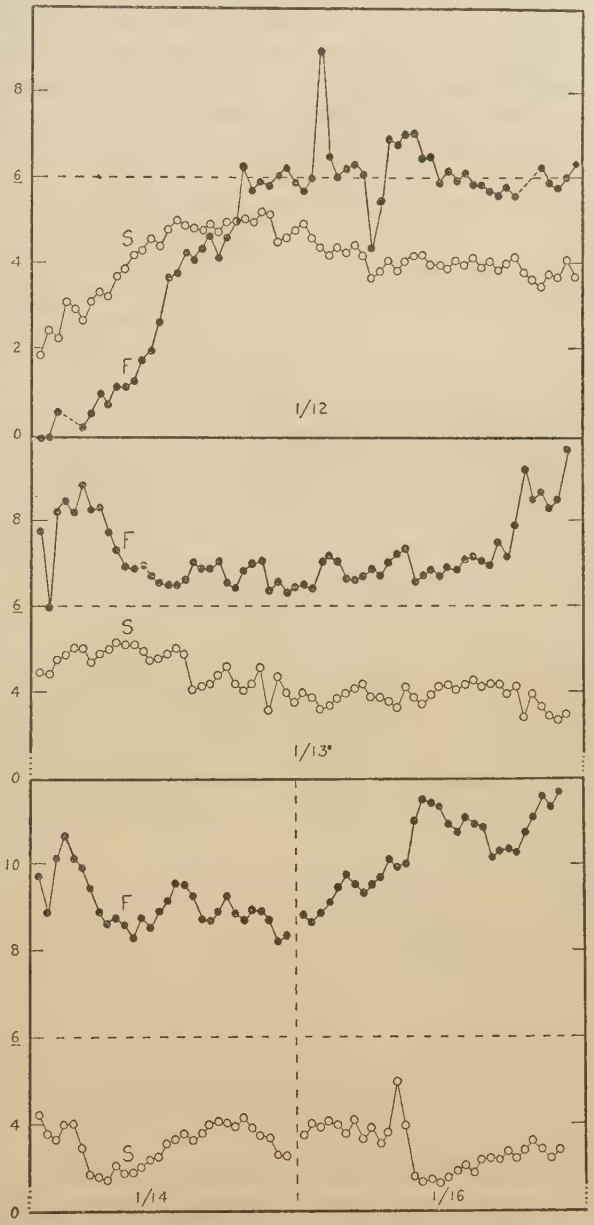

FIGURE 6.—Graph of 5-minute values of *S* and *F* showing progressive adaptation to salinity 28.04 p. p. m. following change from 39.10 p. p. m. For reference the value, *6*, is shown as broken line. Series III. 17°–19° C.

The results of this change to higher salinity are in harmony with other similar changes already described (fig. 7), for the specimen became completely adapted within a few hours, although the initial effect of the change was to reduce the rate of pumping almost to zero. When the salinity was then reduced to 28.17 parts per mille (fig. 8) adaptation during the first few hours was rapid, but it was not until the third day

that the gills were able to pump at the previous rate, even though the valves were well apart.

These tests occupied 10 days, during which time the specimen was highly active and adaptable. At about noon on the tenth day (series IV, table 1) the salinity was reduced to 10.59 parts per mille and a few records kept during the rest of the day. However, the shell remained almost entirely closed. On the next 2 days kymograph records were not made, for the valves were only slightly open and no water was pumped. On the third day the valves had opened wider and the activity was recorded on the kymograph. Only occasionally was a little water discharged either on this day or during the entire 20 days that water of this salinity was used. After the first few days the valves were generally open to a normal degree, and consequently it was the gills themselves that failed to function. The tests were continued for as long as 20 days with the hope that some indication of adaptation of the gill mechanism would appear.

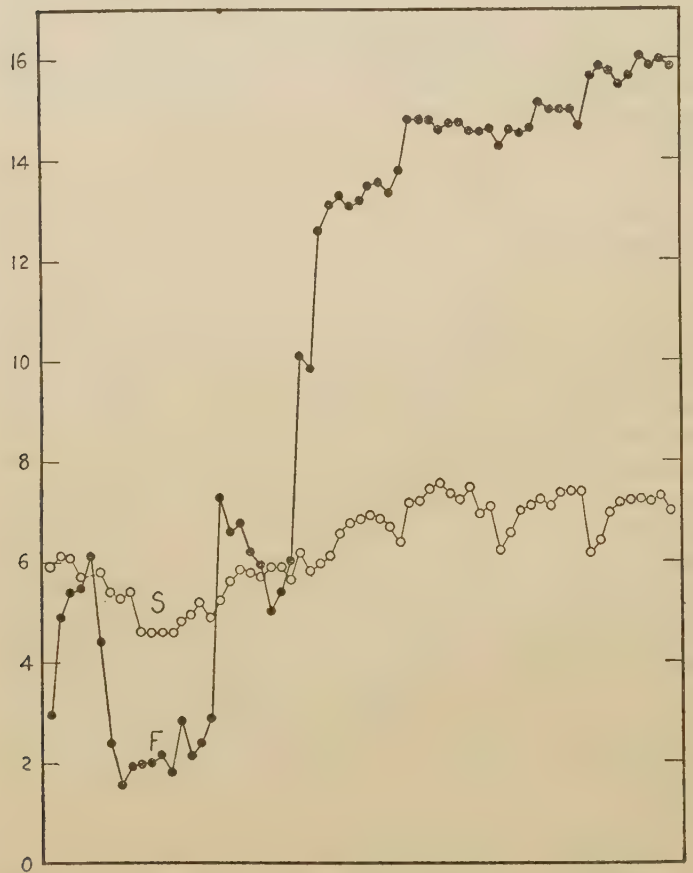

FIGURE 7.—Adaptation of *S* and *F* (5-minute values) to salinity of 36 p. p. m. following change from 28.06. Series IV. 17°–19° C.

In an attempt to locate as exactly as possible the minimum salinity required for functioning of these organs, the salinity was then raised slightly to 12.94 parts per mille and maintained for 4 days. Results being similar to those in the preceding tests, the salinity was again raised to 15.01 parts per mille for 2 days, but without any indication of increased activity. Even when the salinity was raised to 27.92 parts per mille the specimen did not show any sign of recovery. It only remained abnormally wide open, as it has been during many days in water of low salinity. It was clear that subjection to a salinity as low as 10.59 parts per mille for a considerable period of time had done some damage which was not readily repaired even after restoration of more favorable conditions. In fact, it was only after the specimen had been in running sea water for about 2 weeks that it appeared to pump a vigorous stream of water.

Incidentally, the behavior of the oyster during all tests after the introduction of the low salinity was different from the normal in that the quick, partial closures

which occur with characteristic frequency in a normal specimen almost never were to be observed. The oyster acted almost as if anaesthetized, although the shell varied considerably in degree of openness. All movements, however, were slow. Nevertheless, secretion of shell proceeded at a rapid rate, and when the experiment was discontinued several millimeters of thin new shell had been added to the margins of the valves.

SERIES V

In the preceding series the specimen was left in a salinity of 10.59 parts per mille for 20 days, resulting in damage to the pumping mechanism. It was desirable to know whether shorter exposure to such a salinity would have the same harmful effect. A specimen was therefore tested thoroughly for 2 days in water of high salinity (27.35 parts per mille), during which time it reacted favorably, pumping vigorously (series V, table 1). After being changed to a salinity of 10.64 parts per mille, the valves remained at first only slightly open but during the next 2 days opened abnormally wide. As in the previous series, almost no water was pumped. After 4 days of treatment, the salinity was raised to 27.39 and records kept for 5 days. Within about 2 hours after the change the gills began to pump a small stream. Beyond this, however, there was no further recovery during the 5 days. The shell opened wider and wider, but never was more than a feeble circulation of water produced by the gills.

FIGURE 8.—Five-minute values of *S* and *F* during adaptation to a salinity of 28.17 p. p. m. after change from 36 p. p. m. The value, *10*, is shown as broken line for reference. Series IV. 17°–19° C.

The results are similar to those described in series IV, with the difference that in the present series exposure to low salinity had not been sufficiently prolonged to produce the harmful effect to as great a degree.

SERIES VI

With this specimen another attempt was made to obtain comparative data on the reactions to a series of salinities, the changes being made in relatively small steps. The summarized results are shown in figure 9 and table 1, series VI. After 4 days in a salinity of 28.31 parts per mille, during which there was some variability in activity, the salinity was reduced to 23.57 parts per mille and left for 4 days. Adaptation appeared to be quite rapid on the first 2 days; but on the fourth day, for unknown reasons, the value of F was lower than before.

When the salinity was changed to 20.90 parts per mille adaptation was apparently complete on the second day. However, reduction to 16.31 resulted in an entirely different behavior, the specimen becoming adapted within a few hours to a certain low level of pumping which was maintained without improvement during the entire period of the test (6 days). Examination of figure 9, as well as the results of preceding tests, leads to the suggestion that salinities below about 20 parts per mille become more and more strikingly unfavorable in their effect.

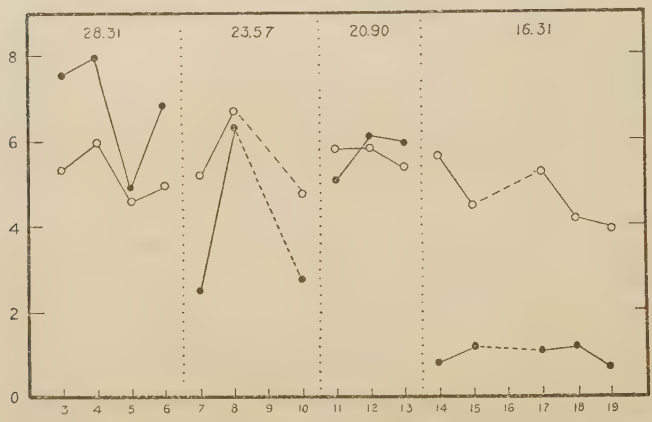

FIGURE 9.—Graph of daily averages of values of S (degree of openness) and F (relative rate of flow) at different salinities. Series VI. 17°–19° C.

Whether adaptation would take place in such instances to the extent that water would be pumped at the same rate as at higher salinities may not definitely be stated, but it is clear that adaptation is extremely slow.

SERIES VII

In those experiments which have already been described it was indicated that when specimens are placed in water of a salinity of about 15 to 17 parts per mille the rate of pumping is greatly reduced and adaptation is very slow, possibly never reaching the level observed at higher salinities. It was also shown that a salinity as low as 10 to 11 parts per mille results not only in almost complete stoppage of the flow of water through the gills but also in a harmful effect from which recovery, after return to high salinity, is extremely slow. It would appear that the lower salinity limit which the oyster can tolerate lies somewhere between 10 and 15 parts per mille. This experiment was performed in order to locate this limit more exactly (series VII, table 1).

Preliminary tests were carried on over a period of 9 days with a salinity of 25.09 parts per mille before changing to a salinity of 13.00 parts per mille. During the first day at the lower salinity the valves did not open wide and there was no flow of water. On the next day it looked as if the specimen was going to become well adapted, but during the following days this rate of activity was not maintained. During 8 days of treatment in water of this salinity only a sluggish stream was pumped as compared with the activity observed during the preceding

tests. The activity in this salinity was only slightly less rapid than that recorded in series I, when a salinity of 14.60 parts per mille was employed. A salinity of about 13 parts per mille appears to be but little more favorable than a salinity of about 10.5 parts per mille as studied in series IV and V. In the latter cases, however, the specimens were not able readily to recover from the harmful effect.

This specimen quickly became adapted to a salinity of 25.53 parts per mille, and in spite of the fact that it had been in the water of lower salinity for 8 days it recovered completely within 2 days. The progress of adaptation in this case is one of the best examples obtained, and a graph (fig. 10) is reproduced showing all of the 5-minute values of S and F. After the first 2 hours, during which the valves were not wide open and almost no water was pumped, the shell consistently opened wider and the rate of flow of water increased. It may be noted that considerable adaptation took place overnight between recordings of the 2 days. This graph suggests also the close correlation between degree of openness and rate of pumping.

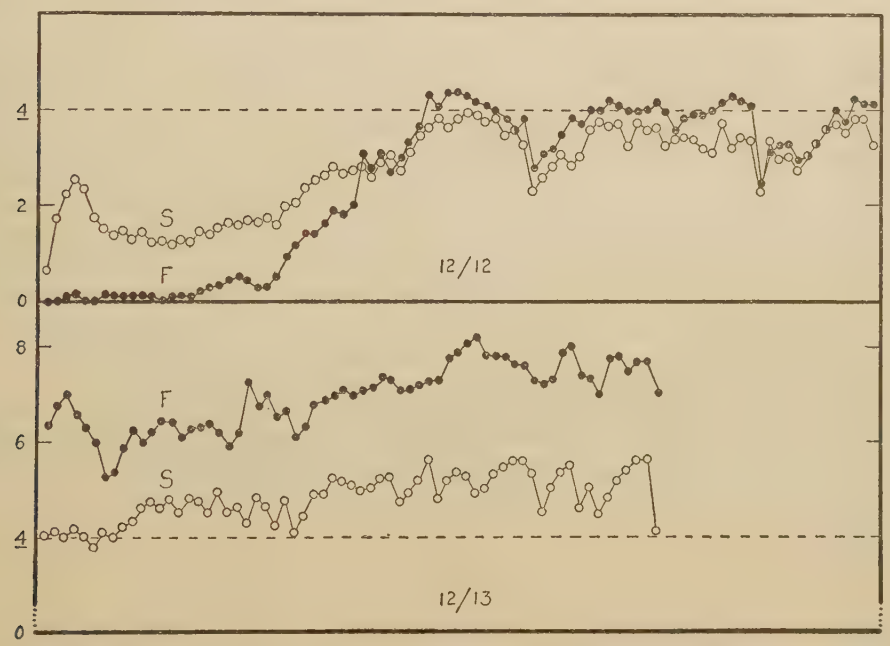

FIGURE 10.—Adaptation of *S* and *F* (5-minute values) to salinity of 25.53 p. p. m. following change from 12.93 p. p. m. Series II. 17°–19° C. The value, *4*, is shown by a broken line for reference.

In many cases of adaptation it is difficult to determine whether the rate of pumping at any time is due to the rate of activity of the gills or to the degree of openness of the valves, which thereby determine how much water may be pumped. By plotting the values obtained during the 2 days when the specimen was in water of a salinity of 25.53 parts per mille, to show the correlation between S and F (fig. 11), it was clearly shown that the points, with a few exceptions due to the initial effect of the change in salinity, fall into definite alinement; that is, the relationship between rate of flow and degree of openness was the same on the first day, when the specimen was beginning to become adapted, and on the second day, when adaptation was almost complete. This is interpreted as direct evidence that adaptation in this case was entirely under the control of the adductor muscle, regulating the

valves, while the gills themselves become adapted quickly. In this respect these results are entirely different from those obtained in series IV and V, in which, after the salinity was raised following treatment in about 10.5 parts per mille, the shells

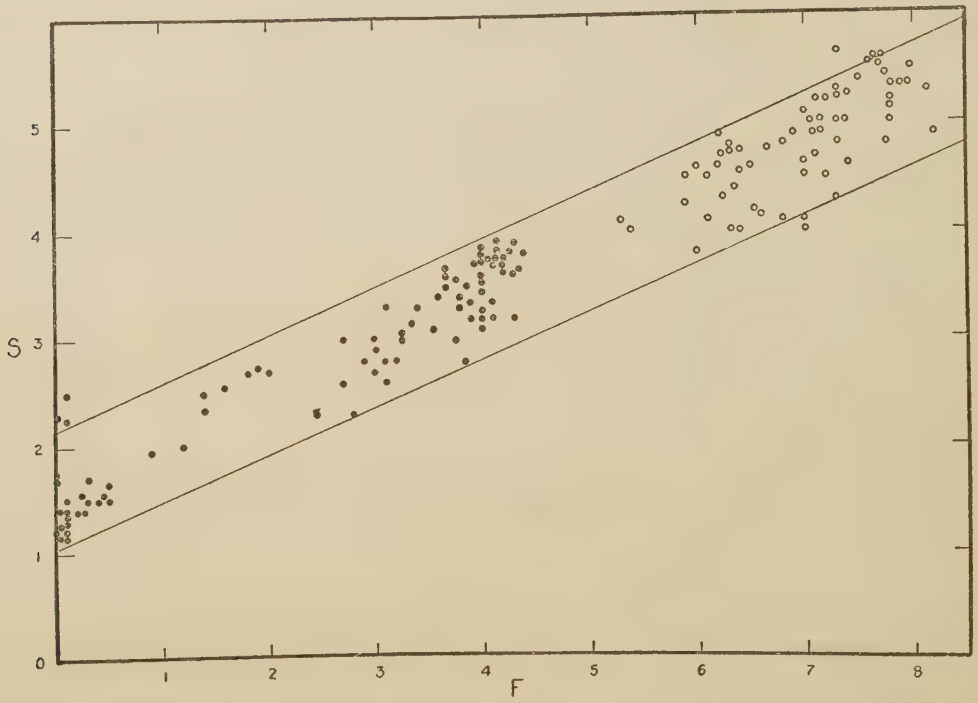

FIGURE 11.—Relationship between S and F during the first (solid points) and second (open circles) days following change in salinity from 12.93 p. p. m. to 25.53 p. p. m. Series II. (See fig. 10.)

remained wide open but the gills were inactive. Between 10.5 and 13 parts per mille, then, there is a tolerance limit, below which there appears to be a destructive effect.

TABLE 1.—*Daily averages of values of S (degree of openness of shell) and F (relative rate of flow of water)*

[17°–19° C.]

SERIES I

Date	Salinity (parts per mille)	Number of 5-minute periods	S (average)	F (average)	Date	Salinity (parts per mille)	Number of 5-minute periods	S (average)	F (average)
Nov 7	29.40	37	*8.53*	*6.45*	Nov. 14	28.50	65	7.99	5.61
Nov. 7	26.96	41	*7.13*	*5.40*	Mean		[1]162	*7.03*	*6.25*
Nov. 8	24.03	47	*7.50*	*6.19*	Nov. 15	22.205	79	*7.63*	*7.62*
Nov. 9	17.76	54	7.42	1.21	Nov. 16	29.46	71	*7.66*	*9.08*
Nov. 10	17.69	47	6.98	5.12	Nov. 18	14.60	125	3.84	.09
Nov. 11	17.69	18	8.15	5.79	Nov. 19	14.60	72	5.35	.70
Mean		[1]119	*7.35*	*3.45*	Nov. 20	14.60	18	7.18	2.15
Nov. 11	28.50	54	5.62	4.67	Nov. 21	14.57	72	6.43	3.43
Nov. 12	28.50	43	7.35	9.20	Nov. 22	14.57	64	7.28	5.17
					Mean		[1]351	*5.48*	*1.93*
					Nov. 23	28.56	51	*6.00*	*4.85*

SERIES II

Nov. 28	28.87	36	*6.33*	*7.29*	Dec. 7	28.94	63	*7.73*	*17.67*
Nov. 29	22.70	71	7.47	5.69	Dec. 8	17.85	29	5.07	.23
Nov. 30	22.70	71	8.17	9.34	Dec. 9	17.85	36	6.98	6.06
Mean		[1]142	*7.82*	*7.51*	Dec. 12	17.83	54	8.83	6.92
Dec. 1	28.74	72	8.38	13.31	Dec. 13	17.83	48	6.08	4.40
Dec. 2	28.74	81	8.73	15.48	Dec. 14	17.83	24	5.67	3.88
Dec. 3	28.74	32	8.60	16.47	Dec. 15	17.83	17	5.65	2.79
Mean		[1]185	*8.58*	*14.31*	Dec. 16	17.83	27	4.77	1.20
Dec. 4	25.08	63	7.47	6.95	Dec. 17	17.83	17	5.96	1.69
Dec. 5	25.08	54	7.80	13.90	Dec. 18	17.83	9	5.22	1.08
Mean		[1]117	*7.62*	*10.14*	Mean		[1]261	*6.43*	*3.98*
					Dec. 19	27.27	53	*7.48*	*12.42*

SERIES III

Jan. 4	28.55	50	*2.89*	*6.44*	Jan. 12	28.04	60	4.15	4.86
Jan. 5	28.27	54	*3.03*	*9.13*	Jan. 13	28.04	63	4.26	7.32
					Jan. 14	28.04	30	4.13	9.73
Jan. 6	39.10	71	2.01	6.30	Jan. 16	28.04	31	4.06	11.04
Jan. 7	39.10	36	2.58	10.01	Mean		[1]184	*4.08*	*7.53*
Jan. 9	39.10	36	3.54	8.72					
Jan. 10	39.10	33	3.57	9.05	Jan. 17	56.32	18	0	0
Jan. 11	39.10	65	3.95	9.10	Jan. 18	56.32	27	1.27	.03
					Jan. 19	56.32	37	1.95	.68
Mean		[1]241	*3.09*	*8.34*	Jan. 20	56.32	10	7.89	.52
					Mean		[1]92	*2.01*	*.33*

SERIES IV

Jan. 25	28.06	50	5.67	9.14	Feb. 15	10.48	18	2.86	0
Jan. 26	28.06	45	7.00	16.70	Feb. 16	10.48	17	8.50	0
					Feb. 17	10.48	51	10.63	.36
Mean		[1]95	*6.60*	*12.44*	Feb. 18	10.48	14	10.97	.22
					Feb. 20	10.48	36	9.46	.38
Jan. 27	36.00	63	6.32	10.32	Feb. 21	10.48	18	5.47	0
Jan. 28	36.00	46	8.15	14.20	Feb. 23	10.48	28	7.08	0
Jan. 29	36.00	51	8.40	12.83					
Jan. 30	36.00	64	8.78	12.75	Mean		[1]398	*7.80*	*.16*
Mean		[1]224	*7.89*	*12.39*					
					Feb. 24	12.94	54	8.38	.19
Jan. 31	28.17	65	8.08	6.21	Feb. 25	12.94	45	11.24	.06
Feb. 1	28.17	64	8.89	10.04	Feb. 27	12.94	28	11.21	.31
Feb. 2	28.17	64	9.12	12.15					
Feb. 3	28.17	18	7.81	7.66	Mean		[1]126	*10.02*	*.17*
Mean		[1]211	*8.61*	*8.85*					
					Feb. 28	15.01	61	11.74	.19
Feb. 3	10.59	14	.38	0	Mar. 1	15.01	55	13.01	.14
Feb. 6	10.59	36	5.57	.40					
Feb. 7	10.59	36	3.42	0	Mean		116	*12.35*	*.17*
Feb. 8	10.59	54	6.74	0					
Feb 9	10.59	36	7.37	0	Mar. 2	27.92	54	11.87	.07
Feb. 11	10.59	17	8.37	0	Mar. 3	27.92	52	12.12	.03
Feb. 13	10.59	23	10.01	.46					
					Mean		[1]106	*12.00*	*.05*

[1] Total.

TABLE 1.—*Daily averages of values of S (degree of openness of shell) and F (relative rate of flow of water)*—Continued

SERIES V

Date	Salinity (parts per mille)	Number of 5-minute periods	S (average)	F (average)	Date	Salinity (parts per mille)	Number of 5-minute periods	S (average)	F (average)
Mar. 7	27.35	61	3.67	7.81	Mar. 13	27.39	54	5.46	1.26
Mar. 8	27.35	61	3.60	7.15	Mar. 14	27.39	54	5.37	1.13
Mean		[1]122	*3.63*	*7.48*	Mar. 15	27.39	63	6.41	.68
					Mar. 16	27.32	51	6.72	.65
Mar. 9	10.64	27	.61	0	Mar. 17	27.32	18	8.48	.65
Mar. 10	10.64	56	5.08	.03	Mean		[1]240	*6.18*	*.90*
Mar. 11	10.64	16	7.18	0					
Mean		[1]99	*4.20*	*.02*					

SERIES VI

Apr. 3	28.31	9	5.36	7.51	Apr. 11	20.90	70	5.80	5.05
Apr. 4	28.31	19	5.91	7.97	Apr. 12	20.90	23	5.88	7.07
Apr. 5	28.31	54	4.55	4.91	Apr. 13	20.90	27	5.41	5.84
Apr. 6	28.31	64	4.96	6.82	Mean		[1]120	*5.72*	*5.62*
Mean		[1]146	*4.95*	*6.30*					
					Apr. 14	16.31	17	5.61	.80
Apr. 7	23.57	44	5.25	2.49	Apr. 15	16.31	72	4.49	1.15
Apr. 8	23.57	25	6.69	6.39	Apr. 17	16.31	52	5.26	1.08
Apr. 10	23.57	43	4.75	2.74	Apr 18	16.31	72	4.19	1.19
					Apr. 19	16.31	27	3.94	.07
Mean		[1]112	*5.46*	*3.46*	Mean		[1]240	*4.58*	*1.07*

SERIES VII

Nov. 24	25.09	94	2.37	2.80	Dec. 7	12.98	32	4.04	.71
Nov. 25	25.09	15	3.54	5.06	Dec. 8	12.98	73	3.40	
Nov. 27	25.09	67	4.52	6.57	Dec. 9	12.93	52	4.49	.39
Nov. 28	25.09	43	4.24	4.97	Dec. 10	12.93	11	3.81	.05
Nov. 29	25.09	55	5.06	6.54	Dec. 11	12.93	80	3.18	.24
Dec. 1	25.09	55	4.21	5.90					
Dec. 2	25.09	55	4.70	4.70	Mean		[1]440	*3.39*	*.42*
Mean		[1]384	*3.98*	*5.04*					
					Dec. 12	25.53	88	2.73	2.51
Dec. 4	13.00	80	1.18	0	Dec. 13	25.53	65	4.80	6.95
Dec. 5	13.00	31	5.71	2.27					
Dec. 6	12.98	81	3.90	.68	Mean		[1]153	*3.61*	*4.40*

[1] Total.

DISCUSSION

Organisms such as oysters which live in inshore waters, bays, and estuaries are necessarily subject to frequent changes in the density or salinity of the medium. The observations of Prytherch (1934) indicate that considerable dilution of sea water with inflowing fresh water containing copper ions is necessary for the successful propagation of the oyster. He also noted that a salinity of from 16 to 18.6 parts per mille is optimum for rate of setting, or attachment, of larvae of *O. virginica*. The natural habitat is thus limited by the salinity of the water, although it may not be assumed that diluted sea water is more favorable for growth and feeding after setting and metamorphosis have taken place. Most frequently, on the other hand, is it to be observed that those grounds which are especially favorable for catching seeds are relatively unsuited for the growing and fattening of oysters for market.

A difficulty of analysis encountered in this work is that even under uniform conditions of temperature and salinity there are marked variations in both rate of activity of the gills and degree of openness of the valves. Such variability is probably to be expected in any study of the reactions of an organism as a whole, for

innumerable factors, both internal and external, influence the activity. Lack of constancy in the rate of pumping of the gills may be traced to contraction or relaxation of the gill musculature, changes in size of the ostia caused by variations in diameter of blood vessels of the gill filaments (Elsey, 1935), secretion of mucus which impedes activity of the cilia, etc. However, in spite of such uncontrolled factors which render exact analysis difficult, the results are of sufficient uniformity to warrant definite conclusions.

The experimental results described above suggest that an important factor influencing the quality of oyster meats grown on different grounds is the salinity conditions. The rate at which the gills pump water, from which food particles are filtered, depends upon the salinity of the medium or, more accurately stated, upon the frequency, amplitude, and duration of changes in salinity. It is well understood that rate of pumping of water does not alone determine the rate of feeding, for the abundance of food material in the water is a variable, depending upon other factors, such as the availability of nitrates and phosphates for utilization by the microscopic plant life on which oysters feed. In some instances land drainage may bring fertilizing materials which cause prolific development of food organisms, at the same time so diluting the sea water that oysters are not able to pump sufficient water and to feed effectively.

Any significant change in salinity causes an immediate slowing or cessation in the rate of pumping. Recovery depends upon the amplitude of the change and upon whether it is to a higher or lower level. While only a few hours may be required for adaptation following a rise in salinity, several days may be necessary for recovery following the same change in the opposite direction. It was thought possible that this difference was due to the fact that the specimens had been grown in water ranging generally about 25 to 29 parts per mille and any change from this level would produce a marked effect, while restoration of this salinity resulted merely in the resumption of normal activity. However, adaptation to a further rise in salinity to 36 or 39 parts per mille, higher than oysters encounter in nature, is also rapid, but when a salinity of about 28 parts per mille is restored adaptation is very slow. Rate of adaptation following a change in salinity is also proportional to the extent of the change. Further, as the salinity becomes lower the sensitivity of the oyster to small changes increases, very much as was found (Hopkins, 1931a) with regard to temperature. It was shown that a small drop in temperature caused closure of the shell if the temperature was well below the optimum but produced no effect if near the optimum.

It appears likely that endosmosis following a reduction in salinity swells the tissues and blood vessels, such as those in the gills, resulting in decrease in the size of the pores, or ostia, through which water is forced by ciliary action. An increase in salinity, on the other hand, would involve extraction of water and consequent increase in size of the ostia. The adductor muscle is probably also affected by swelling and shrinking. The slow rate of adaptation to a lowering of salinity is in most cases clearly traceable to diminished activity of the gills, while the valves soon open as wide as, or wider than, before the change. On the other hand, a rise in salinity involves a slow accommodation in degree of openness of the shells and an almost immediate adaptation of the gill mechanism.

In certain cases there occurred what appeared to be an actual stimulation of gill activity following rise in salinity, whereby the specimen pumped more rapidly

than it had previously pumped in water of the same salinity. With respect to the above interpretation this would possibly be expected, for the ostia would be much wider open than normally after the osmotic pressure of the blood has reached equilibrium with the medium.

Living, as oysters do, in a highly changeable environment, subject to seasonal weather conditions which may cause variations in salinity between almost pure fresh water and undiluted sea water, they must necessarily have a remarkable tolerance for salinity changes. It is well known that in time of freshet oyster beds may be covered for weeks with almost fresh water without resulting in any considerable mortality. That they are able to maintain life under such conditions is probably largely due to their ability to remain closed for long periods of time (Hopkins, 1931b), protecting the actual tissues. However, the present results indicate that pumping of water and feeding probably do not go on under such extreme conditions, so that starvation would eventually take place if the low salinity were of too great duration.

It was shown above that there is a minimum limit of salinity below which pumping does not go on and a further, more lasting effect produced which renders the oyster capable of only very slow recovery after being returned to water of higher salinity. This limit appears to lie between 10.5 and 13 parts per mille. At the latter salinity only a small amount of water was pumped, but recovery was readily accomplished in a high salinity. These results compare favorably with Nelson's (1921) estimate of a salinity of about 10.42 parts per mille as the lowest at which *O. virginica* can feed, although his conclusion was based upon whether or not the shell remained open.

Pumping activity was approximately the same at salinity ranging from about 25 to about 39 parts per mille, although the latter value is considerably higher than that of pure ocean water. A salinity as high as 56 parts per mille is obviously unfavorable, and, although pumping some water, the specimen gaped and would probably have soon died. From an ecological standpoint it is the lower limit that is of importance, for seldom are oysters in nature forced to adjust themselves to a salinity higher than that of the ocean.

The oyster appears to be increasingly sensitive to changes in salinity below about 20 parts per mille, possibly indicating that the optimum is higher. The results do not permit one to state the optimum salinity, but it would appear to be well above 20 parts per mille, possibly as high as 30 or 35 parts per mille. It may be that the optimum depends upon the medium to which the particular oysters have been accustomed for generations, that specimens from beds in relatively fresh bays would react differently from those grown in more saline water, as suggested by Nelson (1923a). Most likely, however, little difference of this nature is to be expected, save that a longer time would be required for adaptation of specimens from the water of higher salinity.

SUMMARY

1. Adaptation of the feeding mechanism of the oyster to changes in salinity was studied by recording on the kymograph the degree of openness of the valves and the relative rate of flow of water pumped by the gills.

2. Both the activity of the gills and that of the adductor muscle, which by controlling the position of the valves determines the size of the inhalent and exhalent apertures, are markedly affected by any considerable change in salinity. The initial effect of such a change is to cause partial or complete contraction of the adductor muscle and slowing or cessation of the flow of water.

3. Recovery, or adaptation, following a rise in salinity is very rapid as compared with adaptation following the same change in the opposite direction. The former may require a few hours, while several days may be necessary in the latter case.

4. The rate of adaptation depends upon the degree of change and upon the extent of departure from optimum salinity. It is probable that, as the salinity departs further from the optimum, adaptation would never be so complete that water would be pumped at the normal rate.

5. Because of the great variability in activity of the gills and in degree of openness even under conditions of constant salinity and temperature, the results do not justify exact statement of the optimum salinity. However, the optimum is probably not greatly different from that of ocean water, for salinities between about 25 and 39 parts per mille appear to produce similar effects.

6. While the oyster will tolerate a salinity as high as 39 parts per mille, higher than that of pure ocean water, and pump at the maximum rate, a salinity of 56 parts per mille, is definitely too high for it to tolerate.

7. As the salinity is reduced below about 20 parts per mille the oyster becomes increasingly sensitive. A longer time is required for adaptation to relative stability, and it is probable that the rate of pumping would never become as high as that observed at salinities of about 28 parts per mille.

8. The lower limit of tolerance, or the minimum salinity at which water is pumped effectively, is between 10.5 and 13 parts per mille. At the former salinity almost no water is pumped, although the valves remain well open.

9. At a salinity of about 13 parts per mille little water is pumped, even after several days are allowed for adaptation, but recovery to normal activity occurs readily following restoration of a higher salinity.

10. A salinity as low as 10.5 parts per mille produces a harmful effect, after which recovery in water of high salinity is extremely slow. This effect appears to be within the gill mechanism rather than the adductor muscle.

11. A change to a lower salinity appears to affect the gill mechanism primarily, while following a rise in salinity the adductor muscle tends to hold the valves closer together than normally, resulting in a slower rate of pumping even though the gills may be well adapted.

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